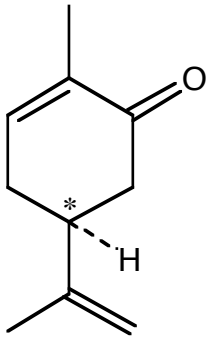
# MASSACHUSETTS INSTITUTE OF TECHNOLOGY

Department of Chemistry
5.310 Laboratory Chemistry

## EXPERIMENT #3
## ESSENTIAL OILS[1]

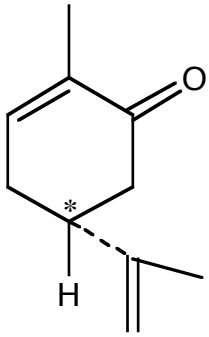
## I. INTRODUCTION

In this experiment[2] you will be working with oils prepared from caraway seeds and spearmint leaves. Each oil has a distinct and characteristic odor, yet carvone is the major component in both oils! It is amazing that the difference in odor is attributable solely to a difference in chirality of the carvone in the two oils. Due to chirality of odor receptors in the nose the R-carvone and S-carvone fit into different receptor sites, hence different odor. Can you distinguish between the odors? 8-10% of the population cannot.[3] Some physical data[4] are presented below.




*(S)* -(+)-Carvone

FW = 150.22; bp 98-100/10 mm
$n_D^{20}$ = 1.4970; d=0.9608 g/mL
$[\alpha]_D^{20}$=+61.7 º (neat 96%)
major component of caraway oil
*(Carum carvi)*




*(R)*-(-)-Carvone

Fw=150.22; bp 227-230 ºC
$n_D^{20}$ = 1.4990; d=0.9593 g/mL
$[\alpha]_D^{20}$=-62.5 º (neat 98%)
major component of spearmint oil
*(Mentha spicata)*

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[1]The experiment includes contributions from past instructors, course textbooks, and others affiliated with course 5.310 updated by John Dolhun May 2017.
[2] Adapted from: Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Engel, R. G. "*Introduction to Organic Laboratory Techniques*"; Saunders: Philadelphia, PA, 1990, pp. 96-107.
[3] *ibid*. p.103.
[4] Physical data is taken from Aldrich Chemical Catalog 1998-1999

All the physical properties should be identical except for the optical rotations of the two isomers (enantiomers), which are of opposite sign. Thus, for both (+)- and (-)-carvone, the infrared, nuclear magnetic resonance spectra, the gas chromatographic retention times, the refractive indexes, and the boiling points should be identical. Hence, the only difference in properties one should observe for the two carvones are the odors and the signs of rotation in a polarimeter. However, some of the physical properties presented above are not identical because of trace impurities.

The * in the formulas above denotes a chiral carbon center. Chiral or asymmetric compounds in nature exist only in living tissue or in matter that was once part of living tissue. Chirality plays a major role in the mechanisms of biological recognition. Yet it is a mystery why caraway plants, *Carum carvi*, produce *S*-(+)-carvone and spearmint plants produce its mirror image *(R)*-(-)-carvone. Other plants such as gingergrass produce racemic carvone. Nature goes one step further; some botanically indistinguishable plants grown in different countries can carry out complete metabolic sequences of mirror-image reactions. Presumably, the enzymes that catalyze the reactions also have a mirror-image relationship. Another example of chiral recognition[5] is found in the effect these two carvone isomers have on rates of reaction. The toxicity of *S*-(+)-carvone in rats is 400 times greater than that of *(R)*-(-)-carvone.

Essential oils are extracts from fragrant plants. They are used extensively in the perfume and flavoring industry. Most components of essential oils are terpenes that contain multiples of a five carbon structural unit, the isoprene unit (Fig. 1).

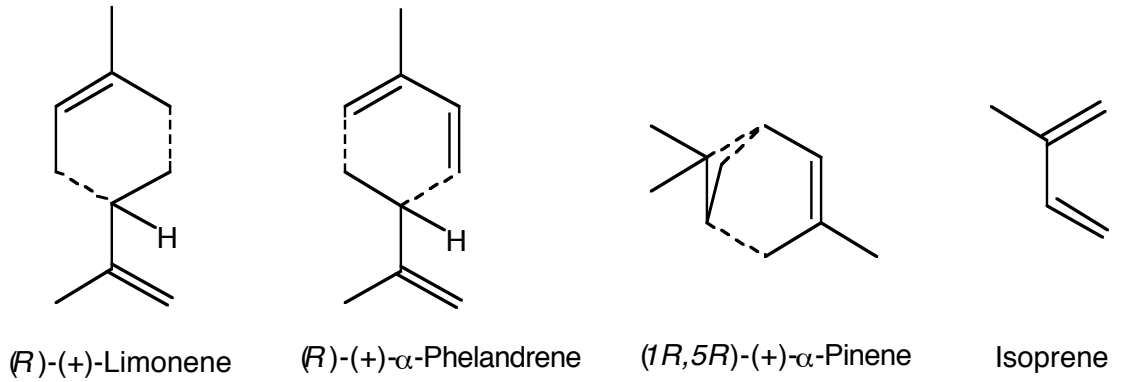



Figure 1. Representative monoterpenes. Isoprene units are shown to indicate the common structural features.

In addition to monoterpenes, compounds derived from two isoprene units, essential oils contain less volatile compounds derived from three and four isoprene units. These higher boiling components will be removed by vacuum distillation of the provided sample to permit facile gas chromatographic separation.

[5] The phenomenon in which a chiral receptor interacts differently with each of the enantiomers of a chiral compound is called **chiral recognition**.

## Overview of the Experiment

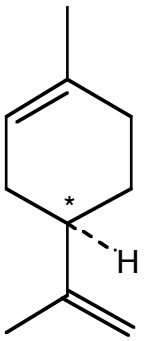
(A) You will be given a sample of either caraway oil or spearmint oil. The major component of these oils is carvone. You will separate the carvone from the higher-boiling and lower-boiling impurities (such as limonene), via vacuum distillation.

(B) You will use gas chromatography and refractometry to check the purity of your distillate and to estimate the relative concentrations of limonene and carvone in the oil.

(C) You will convert the carvone to its semicarbazone for use in a polarimetric analysis.

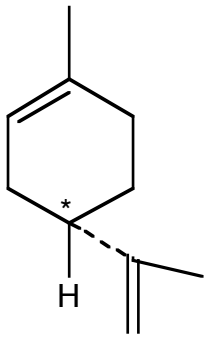
(D) You will obtain infrared spectra of the carvone and limonene fractions and interpret the results.

(E) You will also characterize the semicarbazone by melting point determination.




*(R)*-(+)-Limonene

FW = 136.24; bp 175.5-176$^{o}$C
$n_D^{20}$ = 1.4730; d=0.840 g/mL
$[\alpha]_D^{20}$=+123 $^{o}$ (neat )




*(S)* -(-)-Limonene

Fw=136.24; bp 175-177 $^{o}$C
$n_D^{20}$ = 1.4720; d=0.844 g/mL
$[\alpha]_D^{19}$=-94 $^{o}$ (c=10, ethanol)

(F) Visit the X-Ray Crystallography laboratory where spectra of selected crystals will be determined.

## II. SAFETY

1. **Carvone:** Ketone found in caraway, dill and spearmint oils. The oils are used for flavoring liqueurs, and in perfumes and soaps. Although both enantiomers occur naturally in consumer products, both should be handled with the usual care and not ingested under any circumstances.

2. **Limonene:** Occurs in various oils such as Levant wormseed oil, pine needle oil and other oils. It is used as a solvent, wetting and dispersing agent. It is not considered toxic, but is an irritant. Therefore, keep it off the skin.

3. **Semicarbazide hydrochloride:** Mutagen and cancer suspect agent. Do not inhale or ingest.

4. **Ethanol:** Flammable liquid. The type used in this laboratory is **NOT** safe to drink.

5. **Sodium acetate:** Irritant. Handle with usual caution.

## III. BACKGROUND FOR EXPERIMENTAL PROCEDURE

**General References**

| | |
|---|---|
| • Distillation | **MHS**, Chapter 12, pp 173-205 |
| • Vacuum Pumps | **TM(I)** Sec. 11C. |
| • Gas Chromatography | **MHS**, Chapter 20, pp 291-308 |
| • Polarimetry | **MHS**, Chapter 17, pp 240-251 |
| • Refractometry | **MHS**, Chapter 13, pp 206-211 |
| • Infrared Spectroscopy | **MHS**, Chapter 21, pp 311-344 |

**Videos: Digital Laboratory Techniques Manual**

**#7. Filtration**

**#11. Balances**

**#12. Melting Points**

**#15,16. Distillation I, II**

## Distillation

The difference between the boiling points of carvone (230 °C @ 760 torr) and limonene (177 °C @ 760 Torr) is sufficient to permit separation of the two compounds by distillation. However, carvone thermally decomposes at higher temperatures; therefore, a vacuum distillation is necessary.

Two problems are encountered in a vacuum distillation. The volume of vapor formed from a given amount of liquid is pressure dependent; i.e., the volume of vapor formed from one drop of liquid will be about 30 times as great at 25 torr as it was at 760 torr. As a result, serious bumping may occur. Boiling chips generally do not help much at the reduced pressures. Some of the bumping can be overcome with the use of a magnetic stir bar. The second problem is also related to the larger volume of vapor at lower pressure. The velocity of the vapor entering the column is greatly increased. This creates a greater pressure in the column than is registered on the manometer. Maintaining a slow, steady rate of distillation can minimize this difference in pressure.

## Gas Chromatography and Refractometry

In Gas Liquid Chromatography a mixture of vapors is carried in a stream of helium (carrier gas) through a column. The vaporized sample components move through the column that is lined with a liquid stationary phase. The different components in the sample are retained on the stationary phase for different characteristic relative times. Each component ultimately reaches the Flame Ionization Detector, the most commonly used detector in GC (Air + Hydrogen gas, ratio 10:1). They are detected by their ability to form ions when they are burned in the $H_2$ / air mixture. The area under a peak in a gas chromatogram is proportional to the amount of that substance in the sample.

Among the factors that influence the separation of compounds by gas chromatography are selection of liquid phase, column temperature, and flow rate of carrier gas. Two common liquid (stationary) phases are silicone oil, which separates components on the basis of boiling point, and carbowax (polyethyleneglycols), which separates components on the basis of polarity. The effect of increased column temperature is to decrease the retention time of a component. As a rough approximation, a 10-15 °C decrease in column temperature corresponds to a doubling in the retention time. For most samples, the lower the column operating temperature, the higher the partition coefficient in the stationary phase, and hence, the better the separation. Too low a column temperature can lead to broad, asymmetric peak shapes. The criterion for resolution of the sample is simply achieving baseline separation of the components. Varying the column temperature and selecting the appropriate liquid phase will achieve resolution of the sample into its components. Identification of retention time can be accurately obtained using a pentane peak as a standard. There will always be enough pentane in the syringe to leave a small peak on the chromatogram. The retention time of

the other peaks can be calculated using the pentane peak. The relative amounts of carvone and limonene in each fraction and the original oil may be calculated by using the area under the appropriate peaks.

By measuring the refractive index of the original oil, limonene and carvone fractions, you can estimate the purity of the respective fractions and the composition of the original oil. Assuming that the actual refractive index, $n$, measured for the two-component mixture (limonene and carvone) is linear in the molar fraction, $x$, of any of the components, then one can write:

$$\mathbf{n = (1 - x_{carvone}) * n_{limonene} + x_{carvone} * n_{carvone}}$$

Plug in your data and determine the value of $x_{carvone}$ for the limonene and carvone distillation fractions and the oil itself. Compare these results with those obtained by GC.

## IV. EXPERIMENTAL PROCEDURE

**DAY 1: Distillation and Gas Chromatography: WORK in PAIRS. Split evenly the limonene and carvone fractions for the derivatization step.**

### Part A. Distillation

Before setting up the glassware as shown in Fig. 2 have your teaching assistant demonstrate how to connect to the vacuum pumping manifold. You should be able to reduce the pressure to 1-2 Torr (or less) in a closed system. At the pressure achieved, calculate the temperature at which the limonene and carvone should distill over. Carefully assemble the glassware as shown in Fig. 2. **Lightly grease all joints as demonstrated by your TA**. Be sure to include a heating mantle, a stirring plate and an ice-bath in the setup. **Do not proceed until your TA has checked your setup. Test the vacuum on your system for any leaks without adding sample to the round bottom flask. Why is this important?**

Pour 10 mL per student in your group (i.e. 20 mL for two students; **save** a small portion, about 5 drops, for gas chromatography and refractive index measurements) of your essential oil into the round bottom flask (for 20 mL oil use a 50 mL round bottom flask), and add a 1/2" stir bar.

- Check if the water-in and water-out connections are set correctly (see Fig. 2) and secured with the wire tubing clamp. Make sure you are using water tubing not vacuum tubing.

- Turn the water on in the condenser.

- Immerse only the first receiving flask into a mixture of ice and water until you have collected the limonene then cool all four receiving flask positioned under the outlet tubing of the cow in a mixture of ice and water. Why do you begin only cooling the one flask positioned to collect the first fraction?

- Put aluminum foil around the vigreux column and distilling flask, this will help raise the T of the system making the distillation proceed a bit faster.

- Turn on the magnetic stirrer and fully open the vacuum valve (note: pressure **cannot** be controlled with this valve. If an adjustment is necessary, please see the Instructor). The pressure should read less than 2 mm Hg (2 Torr) but greater than 1 mmHg (1 Torr) on the manometer. Determine the temperature the limonene and carvone fractions should begin to distill based on the pressure reading on the manometer, using the chart at the end of this experiment. Feel free to verify your answers with your TA.

- Use the Variac dial (starting at setting 40); increase the temperature slowly about 5 variac units every 5 minutes. Although some "explosive" bumping is expected, do

not allow the heat to reach such a level that the column is flooded since this will dramatically decrease the efficiency of the separation.

**Note:** On a warm day the room temperature may be at or above the boiling point of limonene (ca 32 ºC) at reduced pressure. If the laboratory is warm be sure to check for limonene distillation before turning on the heat source.

When the oil begins to distill, the condensation can be seen inside the thermometer adapter, thus the progress of distillation can be monitored. The rate at which the temperature changes at the top of the column is significant. Be sure to record temperature information in your lab notebook.

To ensure an optimal separation, three fractions should be collected: **the limonene** fraction, **an intermediate fraction** that forms during the rapid increase in temperature after collection of the limonene fraction, and the **carvone fraction**.

**To collect each fraction, rotate (under vacuum) the cow adapter such that the end of the bent outlet sits above the next empty receiver flask.** Label flasks, for example, with letters **A, B,** and **C**.

When collecting the first fraction, chill only that one flask using a 100 mL beaker filled with ice. After collecting the first limonene fraction then cool ALL four collection flasks simultaneously with a large pyrex crystalizing dish filled with ice for the remaining collections. Why is it now important to cool all four flasks? At the end of the distillation use a glass Pasteur pipette to remove the material from your distillation flasks. Pouring it out will cause your product to be contaminated with stopcock grease, which may result in many extra peaks in your GC trace.

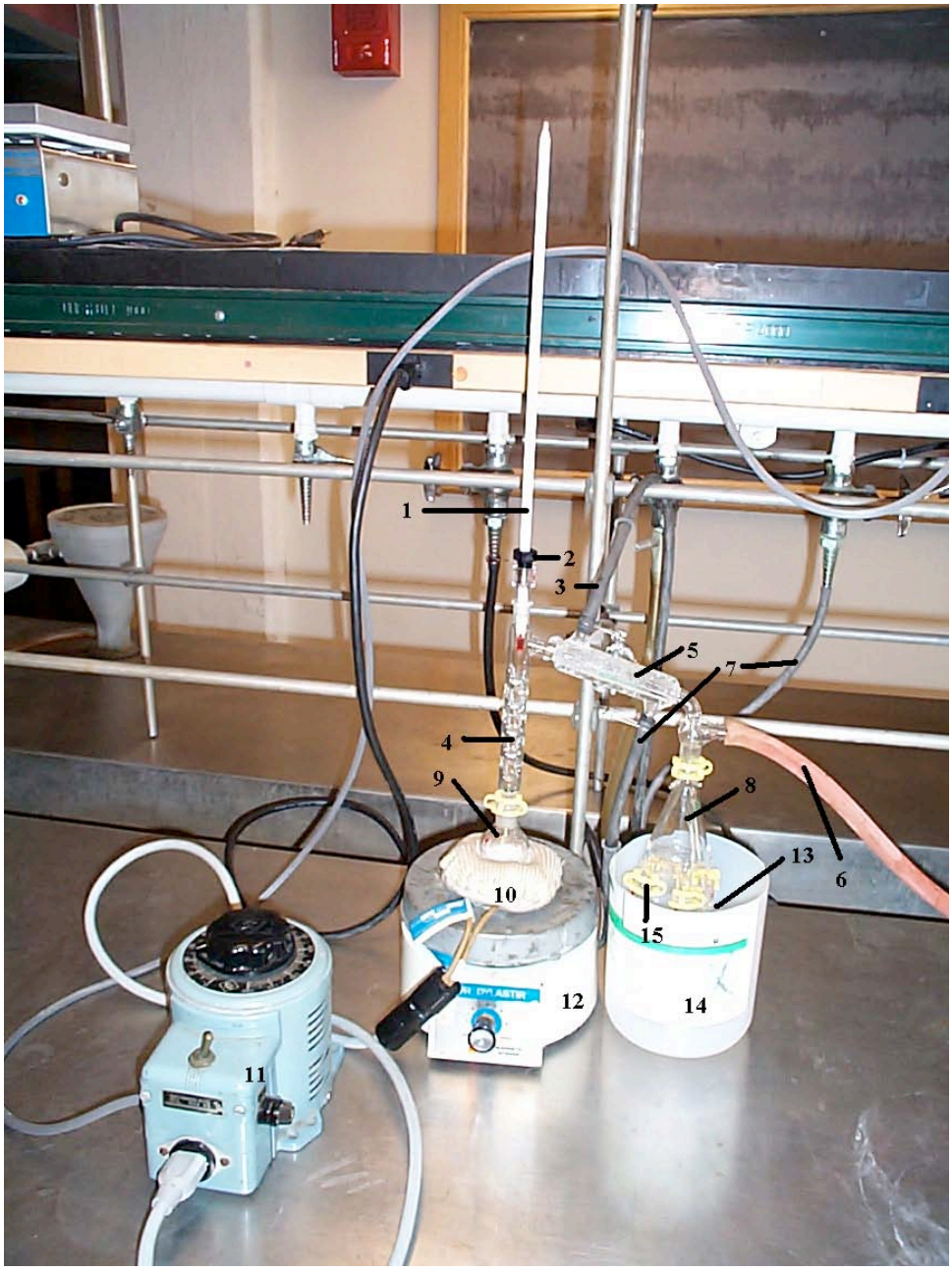



***Figure 2. Apparatus used for Vacuum Distillation***

1. Thermometer
2. Adaptor
3. Water-out
4. Vigreux column
5. Condenser
6. Vacuum connection
7. Water-in
8. Cow
9. 25 mL (10 mL of oil) or 50 mL (15 mL of oil) round bottom flask
10. Heating mantle
11. Variac
12. Stirring plate
13. 10 mL pear-shaped receiving flask
14. Bucket with ice and water
15. Keck clamps

## DAY 2 Part B. Gas Chromatography

Part B. Gas Chromatography: Each team should run the following three GC's:

(a) **the original oil**
(b) **fraction 1 (limonene).**
(c) **fraction 3 (carvone)**.

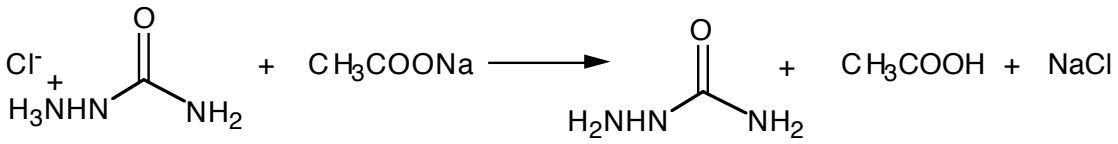
Read the general references on Gas Chromatography in *Techniques in Organic Chemistry* **(MHSM)**. Detailed instructions for use of the Hewlett-Packard 5890-II gas chromatographs are provided in the Appendix at the end of this experiment.

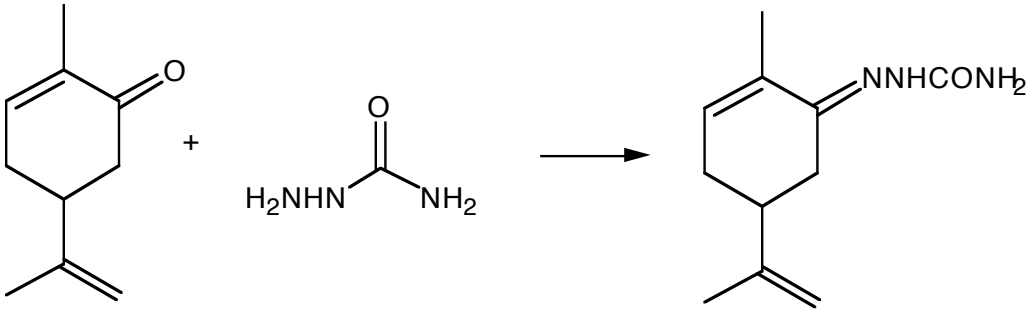
Save a small portion (5 drops) of your original essential oil sample and of the distilled limonene and carvone fractions for Gas Chromatography and Refractive Indexes measurements. The composition of these samples will be analyzed to determine the effectiveness of the separation. Follow the procedures in the appendix **carefully** for preparation of the gas chromatograph samples.

The two biggest problems with sample preparation are:

1) **Not carrying out the double dilution** (use disposable test tubes) for the GC analysis (thus the sample is too concentrated to get clean separation with minimal background noise and may overload the column)
2) **Not placing the barcode label in exactly the right spot** for the instrument to read the label (thus causing the instrument to shutdown). Be sure to record barcode numbers as well as vial positions in the GC line-up.

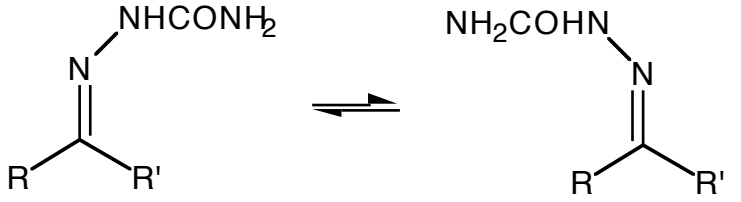
## DAY 2 Part C. Synthesis of Semicarbazone




Carvone | Semicarbazide | Carvone semicarbazone
mp 141-142 °C
FW 207.2

There are two diastereoisomers for the semicarbazone of (-)-carvone and (+)-carvone. They result from the restricted rotation about >C=N- bond. The α-isomer of (-)




or (+)-carvone melts at 162-3 °C, the β-isomer at 141-2 °C. Students will obtain either α-or the β-isomer depending on the actual conditions used. Because the limonene does not have a carbonyl group, the small amount of it, which remains in the carvone fraction, will not form a semicarbazone derivative. The limonene will remain in solution and be washed away during filtration.

In a large test tube, dissolve 0.5 g (4.5 mmole) of semicarbazide hydrochloride and 0.5 g of anhydrous sodium acetate (or 0.8 g of sodium acetate trihydrate) in 4 mL of distilled water and 7 mL of ethanol. Add 0.5 mL (0.48 g, 3.2 mmole,) of your carvone fraction. Stopper and shake your tube vigorously. Remove the stopper; place a boiling

chip in the test tube and place in a beaker of warm water (ca. 80 - 90 °C) for 30 minutes. Careful! Contents of the test tube will boil. Beware of splashing!

There are three ways by which you can isolate the crystals of your semicarbazone derivative:

(1) You can add 3 mL of water and then set the reaction mixture aside to cool slowly at room temperature. Under these conditions, crystallization may take 45 minutes or longer. Slow crystal formation gives quite pure crystals that probably need not be recrystallized for the optical activity studies.

(2) Alternatively, after allowing the reaction mixture to cool for ten minutes or so, you can add three grams of ice or cold water and cool the mixture to 0-5 °C with water-ice bath. If no crystals form, scratch the sides of the flask with a glass rod in order to induce crystallization.

(3) Set up a crystallization apparatus using one of the techniques discussed in lecture.

Collect the crystals by suction filtration using your Hirsch funnel and wash them with a few milliliters of cold water (do not forget to remove the boiling chips, hint: remove boiling chips by decanting the solution before crystallization begins).

Dry the semicarbazone in your desk until the next laboratory session.

## DAY 3 Instrumental Analysis* of the Limonene and Carvone

Be sure to record details about the instruments used manufacturer model (number) for inclusion in your report.

### Part D. Melting Point of Semicarbazone Derivative

Take the melting point of the dry solid semicarbazone derivative and record the yield. If it is necessary to recrystallize the semicarbazone derivative, you may do so from an ethanol/water mixture. Repeat measurement of the melting point to verify the success of the recrystallization.

### Part E. Measurement of Optical Rotation by Polarimetry

Make up 10 mL of a 1.5-3% (w/v) solution[6] of your semicarbazone in ethanol. Add this to a polarimeter tube obtained from the stock room. Measure the optical rotation of the semicarbazone derivative solution in the polarimeter following the procedure demonstrated by your TA. This technique is very dependent on the interaction of light with a clean sample. Be sure the cell is clean. If there is any particulate matter or cloudiness to the solution filter it first. Finally check that no air bubbles have formed which will also interfere with the analysis.

### Part F. Infrared spectroscopy of limonene and carvone

Run infrared spectra following procedure demonstrated by your TA, of the limonene and carvone fractions obtained by distillation. The infrared spectrum may be run using neat liquid on salt plates or as in this experiment, by spotting several drops of liquid (disposable pipet!) on the window area of an IR card. If the peak absorbances are low, place an additional few drops of the limonene or carvone on the IR card, and re-record the spectrum.

### Part G. Refractometry

Measure the refractive indexes of pure limonene, pure carvone, original oil, limonene fraction and carvone fraction following the procedure demonstrated by your TA. Calculate $x_{carvone}$, and compare with the GC data.

[6] Approximately 0.15 g to 0.3 g sample in 10 mL of solution

## DAY 4 X-Ray Crystallography Analysis

Lab sections will visit the MIT Chemistry Department X-Ray Crystallography Laboratory and the crystal structure of selected (+) and (-) carvone enantiomers will be determined.

## Crystal Structure Determination

Crystallography pertains to studying the structure and properties of crystals. More specifically, x-ray crystallography is a method of determining the three-dimensional structure of molecules on the atomic level by means of x-ray diffraction on crystal lattices. The diffraction pattern obtained from the interaction of a monochromatic x-ray beam with the lattice of a single crystal consists of hundreds or thousands of discrete reflections that form a lattice of their own, the reciprocal lattice. The individual reflections in this lattice can be understood as coefficients in a Fourier synthesis where the reflections' intensities correspond to the magnitudes and their reciprocal coordinates translate into the frequency. The result of the Fourier summation is the three-dimensional electron density function of the entire crystal. The determination of a crystal structure consists of several steps all of which pose their individual challenges.

A high quality single crystal is needed to determine a crystal structure and often, **crystal growth** is the bottleneck in structure determination. One of the best methods to grow quality crystals is vapor diffusion: an anti-solvent (also called precipitant) with a higher vapor pressure than the solvent is allowed to diffuse into a vial with a solution of the compound of interest and, over time, crystals form. It is important to keep crystals, once obtained, in their mother liquor as often solvent molecules are incorporated into the crystal lattice and drying the crystals might destroy them.

From the first diffraction images, one can usually judge the quality of the crystal and determine the **unit cell**. Depending on the crystal system, which corresponds to the symmetry group of reciprocal space, a data collection strategy can be devised. A good dataset is complete (>98%) and all data have been collected with a redundancy of 6 or better.

Once all data are collected, correction for polarization and other effects as well as absorption need to me made. This step is called **data reduction** and the result of it is a

file containing a list of all reflections, each with a set of reciprocal coordinates *h*, *k* and *l*, an intensity and a standard uncertainty.

Based on intensity statistics and systematic absences in reciprocal space, the symmetry group of the crystal, the **space group**, can be reconstructed (not always unequivocally). Knowing the space group is vital for correctly determining the crystal structure, as the entire crystal is to be described by (usually) just one or two molecule(s). The crystal structure, therefore, is the spatial average of the entire crystal and space group symmetry operators plus translation expand the structure to the whole crystal.

For a Fourier synthesis one needs magnitude, frequency and phase. Unfortunately, only the first two can be derived directly from the diffraction experiment. Assigning a (preliminary) phase angle to each reflection is called solving the structure. For chemical crystallography, the **phase problem** is solved mostly with direct, dual-space, and Patterson methods; in protein crystallography other methods such as MAD/SAD phasing or molecular replacement are also used.

With the trial phases determined during structure solution, a first Fourier summation is performed and a preliminary model of the molecule can be obtained. In this model, some atom types may be assigned incorrectly and other details of the structure may still be missing. The way from the first solution to the final model is called **structure refinement**. This step can be easy at times (a matter of mere minutes) or difficult in non-routine cases when refinement may take days or sometimes even weeks.

Further Reading:

Müller, P., *Crystallography Reviews* **2009**, *15*, 57-83.

Clegg, W., *X-Ray Crystallography (Oxford Chemistry Primers)* 2nd Edition, Oxford University Press, **2015**.

## V. ANALYSIS & DISCUSSION

**Be sure to incorporate your answers into your written report (discussion section).**

1. Using the refractive index data determines the approximate composition of your oil, your carvone fraction and your limonene fraction with respect to limonene and carvone.

2. From the gas chromatography, report the percentages of limonene and carvone in your original sample and in the fractions following distillation. How effective was the distillation in separating these components? Are there any other (minor) components in any of the fractions? If so, how much of the sample do they constitute? Can you identify any of the minor components? Calculate the # of theoretical plates for each sample peak $n = 5.55\ (t_r/w_{1/2})^2$

3. Compare the results obtained from gas chromatography and refractive index measurements. How closely do they agree? If they are not in good agreement can you provide an explanation?

4. Which isomer of carvone do you have? Is it *R* or *S*? How do you know? What is the association between the chirality and the odor of the carvone isomers?

5. Calculate the **specific rotation** of the semicarbazone derived from carvone,

$$[\alpha]_D^{25} = \frac{\alpha}{C * l}$$

where $\alpha$ is the rotation angle read on the polarimeter, *C* is the concentration of the semicarbazone solution in grams per milliliter of solution, and *l* is the path length of the polarimeter tube in decimeters (these units are employed for historical, rather than rational reasons). Include a propagation of errors!

6. (a) Which features in the IR spectrum, can be assigned to limonene? To carvone? Identify as many functional groups as you can in each sample.

   (b) Do the spectra of the samples suggest the presence of a mixture of these two components, and if so, are they consistent with the GC data?

   (c) Can you use IR spectroscopy to distinguish between *(R)*- carvone and *(S)*-carvone?

7. Your well written discussion should address the following (within the text, NOT as a numerical series of answers to questions):

    1. Comparison of your distillation results (temperature ranges) with your chromatographic results.

    2. Explanation of how the separation of components could be improved.

    3. Comparison of your results (boiling points, compositions, m.p. of semicarbazones, optical rotations) with literature values.

    4. Discussion of what information gas chromatography provides.

    5. Discussion of what information infrared spectroscopy provides.

    6. Discussion of what information X-Ray Crystallography provides.

    7. Discussion of what information these techniques do NOT provide.

    8. Discussion of what the identity of the carvone enantiomer tells you (if anything) about the identity of the limonene in your sample.

## VI. ADDITIONAL READING

1. Ernest L. Eliel and Samuel H. Wilden, "*Stereochemistry of Organic Compounds*"; Wiley: New York, 1994, p 202.
2. S. Robinson and E.R. Gilliland, "*Elements of Fractional Distillation*,"4th ed.; McGraw-Hill: New York, 1950.
3. G. Guiochon, G; Guilleman, C. L. *Gas Chromatography Rev. Sci. Instrum.* **1990**, *61*, 3317.
4. D.L. Sayers and R. Eustace, "The Documents in the Case" (1930): a particularly nasty murder is solved by using polarimetry to distinguish between natural and synthetic muscarine [$C_9H_{18}NO_2$.HCl].
5. "Looking Glass Chemistry," *Science,* **1992**, *256*, 964.

# 5.310 Laboratory Chemistry

Fall 2017